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Monitoring Chinese hamster ovary cell culture by the analysis of glucose and lactate metabolism

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Abstract

Monitoring cell growth is crucial to the success of an animal cell culture process that can be accomplished by a variety of direct or indirect methodologies. Glucose is a major carbon and energy source for cultured mammalian cells in most cases, but glycolytic metabolism often results in the accumulation of lactate. Glucose and lactate levels are therefore routinely measured to determine metabolic activities of a culture. Typically, neither glucose consumption rate nor lactate accumulation rate has a direct correlation with cell density due to the changes in culture environment and cell physiology. We discovered that although the metabolic rate of glucose or lactate varies depending on the stages of a culture, the cumulative consumption of glucose and lactate combined (Q_{GL}) exhibits a linear relationship relative to the integral of viable cells (IVC), with the slope indicating the specific consumption rate of glucose and lactate combined (q_{GL}). Additional studies also showed that the q_{GL} remains relatively constant under different culture conditions. The insensitivity of the q_{GL} to process variations allows a potentially easy and accurate determination of viable cell density by the measurement of glucose and lactate. In addition, the more predictable nature of a linear relationship will aid the design of better forward control strategies to improve cell culture processes.

Keywords: Chinese hamster ovary cells; Cell density; Process monitoring; Metabolism; Glucose; Lactate

1. Introduction

Animal cell culture technologies are widely used in biomedical research and pharmaceutical industry. Assessment of cell growth and metabolic activities are essential to the success in the control and improvement of a cell culture process. In general, the cell mass of a culture can be determined by direct or indirect measurement (Butler, 1999; Stewart et al., 2000; Harms

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et al., 2002). The traditional direct methods such as hemacytometer cell counting or a particle cell counter tend to suffer from variations in sampling and sometimes in measurements. In addition, these techniques offer limited information on metabolic activities of a culture. Whereas indirect methods, such as those based on the measurement of nutrient consumption or metabolite accumulation, can potentially overcome the limitations inherent to the direct methods. In biopharmaceutical manufacturing, both direct and indirect methods are usually used in combination to provide complementary information about a cell culture. For this reason, significant effort has been made to compare and correlate the results from these two types of measurements. Unfortunately, the correlations are rarely linear or linear only at a particular stage of a culture. This is because an indirect methodology, by measuring metabolites, is usually a function of cell density and time, while a direct method measures the cell population at a discreet time point.

Two of the most commonly monitored metabolites are glucose and lactate. Glucose serves as both a main carbon source and an important energy source in most medium formulation. Entry of glucose into glycolytic pathway leads to the formation of pyruvate as the end product. In animal cells, pyruvate can either be shuttled into the TCA cycle or be converted into lactate. Due to the high flux of glucose to pyruvate and the inefficient coupling between glycolysis and TCA cycle, lactate accumulation tends to occur in continuous cell cultures (Hu et al., 1987; Wagner, 1997). Lactate build-up in turn will lead to the acidification of the culture environment. In addition, lactate itself could also be toxic to mammalian cells even under controlled pH (Glacken et al., 1986; Hassell et al., 1991; Ozturk et al., 1992; Lao and Toth, 1997). The accumulation of lactate is often a critical limiting factor of a cell culture process especially when the cell density is high.

In biopharmaceutical manufacturing, monitoring glucose and lactate has become a routine practice due to the simplicity and reliability of measurement as well as their chemical stability in culture medium. More importantly, glucose concentration provides an assessment of energy charge while lactate is considered an important barometer for the accumulation of metabolic byproducts and an indicator of a deteriorating culture environment. As critical culture parameters, glucose and lactate measurements are often the key components in the design for process control in bioreactor operations such as feeding or perfusion strategies (Ozturk et al., 1997; Dowd et al., 1999; Sauer et al., 2000; Altamirano et al., 2004).

Significant effort has been made in the past to correlate glucose and lactate metabolism with cell density. The consumption rate of glucose and the accumulation rate of lactate reflect metabolic activities of cultured cells. If the cellular metabolic activity is constant, the measured metabolic rate will be proportionate to cell density of a culture. In one case where the glucose and lactate levels are controlled through perfusion, a direct correlation between cell density and glucose and lactate metabolic rates has been demonstrated (Ozturk et al., 1997). However, under most cell culture conditions such as a batch culture, the continually changing environment affects cell metabolic activities. Hence it is difficult to establish a correlation between kinetics of a single metabolite and cell density measurement consistent throughout all stages of a batch culture.

In our current studies, we attempt to establish a predictable correlation between the progression of a Chinese hamster ovary cell (CHO) culture and the metabolism of glucose and lactate. The working hypothesis underlying the present work is that lactate produced by the consumption of glucose in CHO cells can be converted back to pyruvate and then proceeds to enter the TCA cycle (Cori, 1931; Harris, 2002). This hypothesis is supported by the observations that lactate levels decrease at low glucose concentrations, presumably taken up for utilization by cells (McKay et al., 1983; Ozturk et al., 1992; Altamirano et al., 2004). Under this hypothesis, glucose and lactate are both considered sources fueling oxidative phosphorylation in CHO cells. We have demonstrated a direct correlation between the cumulative cell mass measured by direct cell counting method and combined glucose and lactate consumption of a culture. The potential implications and applications of this correlation as a way to monitor cell mass changes in animal cell cultures are discussed.

2. Materials and methods

2.1. Cell line and culture medium

A Chinese hamster ovary cell line, DUKXB-11, deficient in dihydrofolate reductase activity (Dr. L.